REMARKS

Applicants amended claims 3-19 and added new claims 20-40. The amendments are made to remove the multiple dependency and more particularly point out and claim the invention. The amendments are fully supported by the application as originally filed and add no new matter. Entry of the amendments is respectfully requested.

Upon entry of the amendment, the application will contain claims 1-40 pending and under consideration. Applicants submit that all of the pending claims are now in condition for allowance. A Notice of Allowance is hereby respectfully requested.

The Examiner is invited to telephone the undersigned, Applicants' attorney of record, to facilitate advancement of the present application.

Respectfully submitted,

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Version with Marking to Show Changes Made

3. (Amended) A human cell [composition] line for use in producing one or more cytokines, prepared by the process [of] comprising:

[(a)] obtaining a parental human cell line capable of producing one or more cytokines; and

[(b)] modifying the cells by introducing a first expression vector comprising: [the] (i) the coding sequence for CrmA operably linked to a first promoter, and [(ii) a first selectable marker-encoding nucleic acid sequence, and (iii)] additional control elements necessary for expression in human cells, into the cells of said cell line; and

[(c) culturing said modified cells in medium containing a first selection agent to select] screening and selecting for CrmA-expressing cells.

4. (Amended) The [A] human cell [composition prepared by the process of] line according to claim 3, [further comprising the step of:] wherein the process further comprising:

[(d)] treating said CrmA-expressing cells in a manner effective to result in enhanced cytokine production, wherein said [transformed] modified and treated cell line is characterized by a level of cytokine production that is at least two times (2X) the level of cytokine production by the corresponding [non-transformed] not-modified parental cell line.

5. (Amended) The [A] human cell [composition prepared by the process of] line according to claim 3, wherein the process further comprising [the step of]:

[(d) further] modifying cells of said parental [said CrmA-expressing] cell line by introducing a second expression vector comprising: (i) the coding sequence for PKR operably linked to a second promoter; and (ii) [a second selectable marker-encoding nucleic acid sequence; and (iii)] additional control elements necessary for expression in human cells, into the cells of said cell line[; and

(e) culturing said further modified cells in medium containing a selection agent specific for said second selectable marker to select for PKR overexpressing cells.], wherein said introduction of said first expession vector to said cells is prior, at the same time or after said introduction of said second expression vector to said cells; and

screening and selecting for PKR overexpressing cells.

6. (Amended) The [A] human cell [composition prepared by the process of] line according to claim 5, [further comprising the step of:] wherein the process further comprising:

[(f)] treating said CrmA and PKR overexpressing cells of said human cell line in a manner effective to result in enhanced cytokine production, wherein said [transformed] modified and treated cell line is characterized by a level of cytokine production that is at least two times (2X) the level of cytokine production by the corresponding [non-transformed] not-modified parental cell line.

7. (Amended) The [A] human cell [composition prepared by the process of claim 4 or] according to claim 6, wherein treating means subjecting said [transformed] modifiedcells to one or both of priming and inducing.

8. (Amended) The human cell [composition] line according to claim 7, wherein priming means exposing said [transformed] modified cells to phorbol myristate acetate (PMA) or interferon-β.

9. (Amended) The human cell [composition] line according to claim 7, wherein inducing means exposing said [transformed] modified cells to a microbial inducing agent selected from the group consisting of Sendai virus, encephalomyocarditis virus and Herpes simplex virus.

10. (Amended) The human cell [composition] line according to claim 9, wherein said microbial inducing agent is Sendai virus.

11. (Amended) The human cell [composition] line according to claim 7, wherein inducing means exposing said cells to at least one non-microbial inducing agent selected from the group consisting of poly(I):poly(C) (poly IC), or poly r(I):poly r(C) (poly rIC), heparin, dextran sulfate, cycloheximide, Actinomycin D, sodium butyrate, a calcium ionophore and chondroitin sulfate.

12. (Amended) The human cell [composition] line according to claim 11, wherein inducing means exposing said cells to polyI:C, cycloheximide and Actinomycin D.

13. (Amended) In an improved method for producing one or more cytokines in human cell culture, the improvement directed to increasing cell viability and the amount of cytokine production, by culturing a parental human cell line under conditions of one or more of (i) modification effective to result in anti-apoptotic protein expression; (ii) modification effective to

result in cytokine regulatory factor overexpression; (iii) priming; and (iv) inducing, wherein the amount of cytokine production is at least two times (2X) the level of cytokine production by the corresponding [non-transformed] not-modified parental cell line.

14. (Amended) The method according to claim 13, wherein modification effective to result in anti-apoptotic protein expression means introducing a first expression vector comprising: [the] (i) the coding sequence for CrmA operably linked to a first promoter, and (ii) [a first selectable marker-encoding nucleic acid sequence, and (iii)] additional control elements necessary for expression of CrmA in human cells into the cells of said cell line[, and culturing the cells in medium containing a first selection agent to select for CrmA-expressing cells.]; and screening and selecting for CrmA-expressing cells.

15. (Amended) The method according to claim [14] 13, wherein modification effective to result in cytokine regulatory factor overexpression means introducing a second expression vector comprising: (i) the coding sequence for PKR operably linked to a second promoter[; (ii) a second selectable marker-encoding nucleic acid sequence; and (iii)] and (ii) additional control elements necessary for expression of PKR in human cells into cells of said [CrmA-expressing] cell line, [and culturing the cells in medium containing a selection agent specific for said second selectable marker to select for PKR-overexpressing cells.] wherein said introduction of said first expression vector to said cells is prior, at the same time, or after said introduction of said second expression vector to said cells; and screening and selecting for PKR-overexpressing cells.

16. (Amended) The method according to claim [14 or 15] 13, [further comprising] wherein priming [the cells by] means exposing [them] cells of said cell line to one or both of phorbol myristate acetate (PMA) and interferon-β.

17. (Amended) The method according to claim [15 or 16] 13, [further comprising] wherein inducing means [the cells by] exposing [them] cells of said cell line to a microbial inducing agent selected from the group consisting of Sendai virus, encephalomyocarditis virus and Herpes simplex virus.

18. (Amended) The method according to [any one of claims 16 or 17] claim 13, wherein, [further comprising] inducing means of exposing cells of said cell line [the cells by exposing them] to at least one non-microbial inducing agent selected from the group consisting of

poly(I):poly(C) (poly IC), or poly r(I):poly r(C) (poly rIC), heparin, dextran sulfate, cycloheximide, Actinomycin D, sodium butyrate, a calcium ionophore and chondroitin sulfate.

19. (Amended) The method of according to claim 13 [any one of claims 17 or 18], wherein the one or more cytokine(s) are selected from the group consisting of interferon-alpha (IFN-alpha), interferon-beta (IFN-beta), interferon-gamma (IFN-gamma); granulocyte macrophage colony stimulating factor (GM-CSF); granulocyte colony stimulating factor (G-CSF); interleukin-2 (IL-2); interleukin-3 (IL-3); interleukin-7 (IL-7); interleukin-8 (IL-8); interleukin-10 (IL-10); and interleukin-12 (IL-12).

20. The method according to claim 17, wherein said microbial inducing agent is Sendai virus.

21. The method according to claim 18, wherein said non-microbial inducing agent are polyI:C, cycloheximide and Actinomycin D.

22. The method according to claim 14, wherein said first expression vector further comprises a first selectable marker encoding nucleic acid sequence; and wherein said screening and selecting for CrmA-expression cells mean culturing said modified cells in medium containing a first selection agent to select for CrmA-expressing cells.

23. The method according to claim 15, wherein said second expression vector further comprises a second selectable marker encoding nucleic acid sequence; and wherein said screening and selecting for PKR overexpressing cells mean culturing said modified cells in medium containing a second selection agent to select for PKR overexpressing cells.

24. The method according to claim 14, wherein said parental human cell line is also capable of expressing PKR, and wherein modification effective to result in cytokine regulatory factor overexpression comprises screening and selecting for PKR overexpressing cells that exhibits at least a 2-fold (2X) increase in PKR activity, expression and/or production.

25. The human cell line according to claim 4, wherein treating means subjecting said modified cells to one or both of priming and inducing.

26. The human cell line according to claim 25, wherein priming means exposing said modified cells to phorbol myristate acetate (PMA) or interferon-β.

27. The human cell line according to claim 25, wherein inducing means exposing said modified cells to a microbial inducing agent selected from the group consisting of Sendai virus, encephalomyocarditis virus and Herpes simplex virus.

28. The human cell line according to claim 27, wherein said microbial inducing agent is Sendai virus.

29. The human cell line according to claim 25, wherein inducing means exposing said cells to at least one non-microbial inducing agent selected from the group consisting of poly(I):poly(C) (poly IC), or poly r(I):poly r(C) (poly rIC), heparin, dextran sulfate, cycloheximide, Actinomycin D, sodium butyrate, a calcium ionophore and chondroitin sulfate.

30. The human cell line according to claim 29, wherein inducing means exposing said cells to polyI:C, cycloheximide and Actinomycin D.

31. The human cell line according to claim 3, wherein said parental human cell line is also capable of expressing PKR, and wherein the process further comprises screening and selecting for PKR overexpressing cells that exhibit at least a 2-fold (2X) increase in PKR activity, expression and/or production.

32. The human cell line according to claim 31, wherein the process further comprising:

treating said PKR overexpressing cells in a manner effective to result in enhanced cytokine production, wherein said modified and treated cell line is characterized by a level of cytokine production that is at least two times (2X) the level of cytokine production by the corresponding not-modified parental cell line.

33. The human cell line according to claim 32, wherein treating means subjecting said modified cells to one or both of priming and inducing.

34. The human cell line according to claim 33, wherein priming means exposing said modified cells to phorbol myristate acetate (PMA) or interferon-β.

35. The human cell line according to claim 33, wherein inducing means exposing said modified cells to a microbial inducing agent selected from the group consisting of Sendai virus, encephalomyocarditis virus and Herpes simplex virus.

36. The human cell line according to claim 35, wherein said microbial inducing agent is Sendai virus.

37. The human cell line according to claim 33, wherein inducing means exposing said cells to at least one non-microbial inducing agent selected from the group consisting of poly(I):poly(C) (poly IC), or poly r(I):poly r(C) (poly rIC), heparin, dextran sulfate, cycloheximide, Actinomycin D, sodium butyrate, a calcium ionophore and chondroitin sulfate.

38. The human cell line according to claim 37, wherein inducing means exposing said cells to polyI:C, cycloheximide and Actinomycin D.

39. The human cell line according to claim 3, wherein said first expression vector further comprises a first selectable marker encoding nucleic acid sequence; and wherein said screening and selecting for CrmA-expression cells mean culturing said modified cells in medium containing a first selection agent to select for CrmA-expressing cells.

40. The human cell line according to claim 5, wherein said second expression vector further comprises a second selectable marker encoding nucleic acid sequence; and wherein said screening and selecting for PKR overexpressing cells mean culturing said modified cells in medium containing a second selection agent to select for PKR overexpressing cells.